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DECONTAMINATION OF COMBAT WOUNDS IN THE INJURED SOLDIER

FINAL REPORT

by

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Under this contract investigations in wound management have not only identified the potentiators of infection but also improvements in the therapy which will reduce the incidence of infection. In the area of infection potentiators, our studies have included soil, surgical drains, dead space, implants, vasoconstrictors, devitalized tissue, and the damage from impact. In the area of improvements to wound care, our studies have included mechanical cleansing, high pressure irrigation, topical proteolytic enzymes, improved bacteriological

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monitoring techniques, faster antibiotic sensitivity testing, and improved antiseptic agents. In addition to these detailed studies, there were associated studies which explored problems that were also of benefit to the civilian population. In this category were studies on skin grafting, amputations, cellulitis and gangrene, and burns.

FOREWORD

In conducting the research described in this report, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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INTRODUCTION

The fate of traumatic soft tissue wounds is held in a delicate balance between the host's resistance to infection and the causal factors of infection. In order to reduce wound infection, these causal factors have to be identified and understood. During the period June 1, 1972 to October 31, 1979, the U.S. Army Medical Research and Development Command has supported our attempts to improve the management of the traumatically injured combat soldier.

These investigations in wound management have not only identified the potentiators of infection but also improvements in the therapy which will reduce the incidence of infection. In the area of infection potentiators, our studies have included soil, surgical drains, dead space, implants, vasoconstrictors, devitalized tissue, and the damage from impact. In the area of improvements to wound care, our studies have included mechanical cleansing, high pressure irrigation, topical proteolytic enzymes, improved bacteriological monitoring techniques, faster antibiotic sensitivity testing, and improved antiseptic agents. In addition to these detailed studies, there were associated studies which explored problems that were also of benefit to the civilian population. In this category were studies on skin grafting, amputations, cellulitis and gangrene, and burns.

The results from these studies have been presented at numerous medical meetings, conferences, and seminars. In addition, these results have been published in the medical

literature as 29 formal articles. This report will represent a brief review of these contributions which resulted from the funding supplied by the U.S. Army Medical Research and Development Command.

REVIEW OF RESEARCH PROJECTS
(June 1, 1972 to October 31, 1979)

WOUND INFECTION POTENTIATORS

Soil

Even the most trivial combat wound is regularly contaminated with soil. Unless this foreign body is adequately cleansed from the wound, the risk of subsequent infection is great. The causal factors for this deleterious effect of soil have, until now, been unknown. Results of this study have led to the identification of the factors in soil that potentiate the development of infection.

When an injury contained as few as 100 bacteria, the addition of 5 mg of soil led to infection. When this soil sample was fractionated, it was found that the infection-potentiating factors (IPFs) resided predominantly in the clay or organic soil fractions or both. The type of cation adsorbed by the surface of the IPF did not influence its toxic effects on tissues. Silicate clay fractions contain several colloidal clay minerals: montmorillonite, illite, and kaolinite. Montmorillonite clay enhanced the development of infection more than did illite or kaolinite.

The IPFs in organic and clay fractions of soil can be characterized by their large surface area and high cation

exchange capacity. This physical property of the IPFs ensures an active chemical exchange between the wound and the IPFs which may account for their deleterious effects. Isolation and identification of the factors in soil that potentiate infection will enable us to develop techniques for the efficient removal of these agents based on modern pedologic methods.

Investigations into the mechanism of this deleterious effect showed that the body's primary defense mechanism is totally inhibited in the presence of soil. Even when antibiotics are used to prevent the development of infection, they are ineffective because of chemical inactivation due to complexation with the reactive soil particles.

Surgical Drains

The presence of drains in experimental wounds contaminated with a subinfective dose of bacteria damaged the host's defenses. The type of material employed in the drain construction played an insignificant role as a determinant of infection. All drains tested (Silastic and Penrose latex) dramatically enhanced the infection rate of tissue to a similar degree. The mechanism by which drains enhanced infection appeared to be secondary to the drain itself. On the basis of the studies, we believe that the prophylactic use of drains in wounds or cavities when no obvious drainage exists should be incriminated.

Dead Space

Dead space in wounds may result from either tissue loss or failure to approximate tissue. With a reproducible

experimental model, suture closure of dead space was found to enhance the incidence of infection. The deleterious effects of suture closure of dead space appear to be secondary to the foreign body in the wound. Surgical sutures impair the wound's ability to resist infection and eliminate any benefits of dead space closure.

Implants

The role of implant porosity as a determinant of infection was studied. Standardized porous and nonporous polymethyl methacrylate implants were fabricated for this investigation. In rabbits, the premolded polymethyl methacrylate implants were placed under the paravertebral fascia, just superficial to the paravertebral muscle fascia, before inoculation with a measured number of staphylococci. The polymethyl methacrylate implants did not enhance the infection rate of the contaminated wounds. The presence of pores within the implants did not damage tissue defenses. Polymethyl methacrylate implants did not alter the success of antibiotic treatment. Antibiotics prevented the development of infection in wounds containing 10^6 organisms, even in the presence of an implant. When higher levels of organisms were delivered to the wound, antibiotic treatment had a negligible benefit in the implant and control wounds.

Vasoconstrictors

Epinephrine impairs the wound's ability to resist infection. The deleterious effect of this powerful local vasoconstrictor is proportional to its concentration. Concentrated solutions of epinephrine (1:30,000 and 1:100,000) markedly potentiated the development of infection. The infection rates

of wounds subjected to these concentrated solutions were significantly greater than the control wounds. Necrotic skin tissue was commonly encountered in the central portions of the inoculation sites receiving concentrated solutions of epinephrine. The indurate wound margins and the bacterial counts of the contaminated wounds subjected to concentrated solutions of epinephrine were significantly higher than those of the control contaminated wounds.

The inflammatory responses of the contaminated wounds to the more dilute solutions of epinephrine (1:200,000, 1:400,-000, and 1:800,000) were less than to the concentrated solutions of epinephrine. However, it is important to note that the inflammatory responses to the dilute solutions of epinephrine were still greater than to saline. The indurated margin of the contaminated wounds subjected to epinephrine was significantly wider than that of the control wounds. While the number of S. aureus recovered from wounds treated with epinephrine and the control wounds did not differ significantly, infection was detected in a substantial number of the epinephrine treated wounds. It is important to point out that no infection was encountered in the control wounds.

The addition of a 2% lidocaine solution (Xylocaine) to the 1:800,000 solution of epinephrine appeared to minimize the inflammatory response to epinephrine. In these wounds, as well as the control wounds, no gross infection or induration was detected. The contaminated wounds subjected to more dilute solutions of 1% lidocaine with epinephrine (1:400,-000 and 1:800,000) resulted in a significant amount of wound

induration as compared to the control wounds.

The preservative, sodium meta-bisulfite, employed in solutions containing epinephrine, did not impair tissue defenses. The inflammatory response of contaminated wounds to this reducing agent did not differ from that to saline.

Devitalized Tissue

The harmful influences of devitalized tissue on wound defenses are documented and the importance of wound debridement in the care of the traumatic wound is stressed. All devitalized soft tissues damaged the host's defenses and encouraged the development of infection. The capacity of devitalized muscle, fat, and skin to enhance infection was comparable. The infection-potentiating effect of skin was enhanced by exposing it to a dry thermal injury. The mechanisms by which devitalized soft tissue enhanced infection are several. The devitalized soft tissue acts as a culture medium promoting bacterial growth. In addition, the devitalized tissue inhibits leukocyte phagocytosis of bacteria and subsequent kill. Finally, the anaerobic environment within the devitalized tissue may also limit leukocyte function.

Blunt Trauma

A standardized experimental impact injury model was developed to produce soft tissue trauma that simulated impact injuries to soft tissue overlying the cranium. An aluminum impact instrument was constructed so as to deliver a measured amount of energy to a finite area of soft tissue over a fixed

foundation. Impact injury resulted in readily demonstrable changes in the morphology of the tissue and its blood flow. As measured by the distribution of fluorescein dye, the blood flow to the impact site was considerably less than that to unwounded tissue. The magnitude of this reduction in blood flow to the site of injury was proportional to the level of energy absorbed by the tissue. The injury to skin resulting from the impact was restricted primarily to the subcutaneous tissue and panniculus carnosus. Loss of adipose tissue and necrosis of skeletal muscle fibers in these layers were associated with the development of a dense inflammatory infiltrate. The structural damage to the tissue and its reduced perfusion were correlated with the tissue's increased susceptibility to infection by bacteria delivered either by direct infection or as a result of a bacteremia. The magnitude of this damage to the host's defenses is, again, directly related to the amount of energy absorbed per unit area of soft tissue. Antibiotics did suppress the growth of bacteria in these experimental soft tissue impact wounds, even when treatment was delayed eight hours. While the therapeutic efficacy of antibiotics was readily apparent in soft tissue impact wounds, the degree to which they could suppress bacteria in these wounds was less than that encountered in the treated control wounds not subjected to impact.

THERAPEUTIC IMPROVEMENTS

Rapid Bacterial Quantitation

An improved technique has been developed to predict rapidly the critical number of bacteria in tissues. This technique gave reliable and accurate measurements for wounds containing more than 2×10^5 organisms per gram of tissue. It is fortuitous that 10^6 bacteria is the critical number of bacteria that will induce clinical infection in experimental animals and humans. The rapid slide technique is now being utilized routinely in our medical center to determine graft bed receptiveness, predict the safety of wound closure, and monitor the course of burns.

Rapid Antibiotic Sensitivities

This study was undertaken to develop a more rapid antibiotic sensitivity test of the pathogens in soft tissue infections and burn wound eschar. The proposed rapid antibiotic sensitivity test was performed directly on the clinical specimen rather than on single strains of bacteria isolated from the tissue. By performing the antibiotic sensitivity tests directly on the tissue sample, the physician obtains the test results within seven hours after receiving the specimen rather than 38 to 52 hours later, the time delay encountered with the conventional technique. The modification used did not alter most of the standards advised by the Food and Drug Administration, since there was no inclusive change in medium, agar depth, or antibiotic sensitivity disk.

The changes in the Kirby-Bauer test necessitated by using the clinical specimen did not alter significantly the interpretation of the antibiotic susceptibility. If clinically significant numbers of bacteria, 10^6 - 10^9, were present, variations in the inoculum size did not appreciably change the results of the antibiotic susceptibility tests. If the incubation requirement for the standard Kirby-Bauer antibiotic susceptibility test was reduced to seven hours, it also did not significantly limit the accuracy of the test.

As expected, the variable most difficult to standardize was the heterogeneous inoculum containing large numbers, 10^7, of different organisms. A zone of inhibition interpreted as sensitive with one organism was occasionally masked by the presence of the confluent growth of an organism in which the zone of inhibition was considered resistant. However, even in this instance, it is possible that mixed culture sensitivities may provide the most valid information in mixed infections, since they more closely simulate the real clinical situation.

Mechanical Cleansing

Mechanical cleansing of a wound with a sponge soaked in a surfactant has prevented the development of experimental wound infection. The surfactant utilized for wound cleansing is Pluronic F-68, a member of a family of block copolymers called Pluronic polyols. Long-term toxicity studies and clinical trials suggest that this surfactant is safe for human use. Pluronic F-68 is a nonionic detergent that does n

have any intrinsic antibacterial activity.

Although mechanical cleansing with saline-soaked sponges effectively removes bacteria, it damages the wound and impairs its resistance to infection. The severity of the damage to the skin exerted by the sponge can be correlated with its porosity. Sponges with a low porosity are abrasive and exert more damage to skin than do sponges with a higher porosity. The addition of Pluronic F-68 to even the most abrasive sponges ensures that the bacterial removal efficiency of the sponge scrub is maintained, while tissue trauma is minimized. This dual effect of the surfactant results in a dramatic reduction in the infection rate of contaminated wounds. On the basis of these results, a clinical trial with surfactant-soaked sponges would appear to be indicated.

High Pressure Irrigation

All traumatic wounds are contaminated to some degree by both soil and bacteria. Specific infection potentiating factors in soil impair the defenses of the tissue and invite infection. These factors are small in size and resist removal by low pressure irrigation. The efficiency of wound irrigation is markedly improved by delivering the irrigant to the wound under continuous high pressure. Irrigation of the wound with saline solution delivered at 15 pounds per square inch removed 84.8 per cent of the soil infection potentiating factors from the wound. The residual infection potentiating factors remaining in the wound did not significantly impair tissue defenses. On the basis of these experimental studies, clinical studies are now being initiated to test

the therapeutic value of high pressure irrigation in traumatic wounds in humans.

This study was undertaken to identify side-effects of high pressure irrigation. Standardized surgical wounds made in Yorkshire pigs were subjected to high pressure syringe and pulsatile irrigation. As a result of these treatments, fluids were disseminated into the adjacent tissue of the wound, predominantly in a lateral direction. Bacteria did not accompany this disseminated fluid and apparently were filtered out by the surface tissues. This treatment results in a tissue injury which impairs its defenses, making the wound more susceptible to infection. However, the remarkable cleansing capacity of high pressure irrigation appears to outweigh this side-effect, since heavily contaminated wounds subjected to this treatment heal per primum without infection.

Proteolytic Enzymes

When a tissue is injured, its vessels exhibit a marked increase in a vascular permeability. Blood proteins, including fibrinogen, traverse the vessel walls and lead to the development of a surface coagulum. This inflammatory response continues until primary closure of the wound edges is accomplished. The thickness of the surface coagulum is roughly proportional to the time interval between wounding and closure. This coagulum encompasses the surface contaminants, preventing contact with either topical or systemic antibiotics. The presence of this surface

coagulum limits the time in which antibiotic prophylaxis is effective. At three hours after injury, antimicrobial prophylaxis of contaminated wounds has no therapeutic value.

Hydrolysis of the protein coagulum by proteolytic enzymes enhances the activity of the antibiotic in experimental wounds. The success of proteolytic enzymes as adjuncts to delayed antibiotic treatment can be correlated with the clot lysis activity of the enzymes in vitro. Travase, the most potent fibrinolytic enzyme, is the most effective adjunct to delayed antibiotic therapy of contaminated wounds. In contrast, the active enzymes found in Elase, which exhibit no significant clot lysis activity in vitro, do not potentiate the activity of antibiotics in wounds subjected to a delay in treatment.

Travase prolongs the period of effective topical antibiotic action for at least eight hours in experimental contaminated wounds. The therapeutic merit of Travase is also apparent when the antibiotic is administered systemically. Travase shows promise as an adjunct to a variety of antibiotics that are effective against both gram-positive and gram-negative organisms.

The results of these experimental studies support our belief that clinical studies should now be initiated to test the therapeutic value of Travase as an adjunct to antibiotics in heavily contaminated wounds subjected to an unavoidable delay in treatment.

Antiseptic Agents

Iodophors are effective germicidal agents that have prolonged antiseptic activity in contaminated wounds. A nontoxic surfactant, Pluronic F-68, has been used to formulate a safe and effective iodophor. The parameters necessary to regulate the activity of the iodophor were studied to develop a potent, yet safe bactericidal solution for use in human subjects.

The parameters found to be most important were the pH of the solution and the concentration of sodium iodide. Lowering the pH of iodophors increased their stability and antiseptic activity. The free iodine in iodophor solutions prepared with a low pH is predominantly the highly biocidal diatomic iodine (I_2). The concentration of iodide regulated the equilibrium of the dissolved iodine between its free and complexed form. Increasing the concentration of iodide in the iodophor lowered the amount of free iodine in solution and enhanced the concentration of the complexed iodide. It is the level of free iodine in an iodophor that determines its antiseptic activity. Low levels of free iodine yielded iodophors that had a slow bacterial kill rate but a prolonged duration of action. Manipulation of these variables permitted the generation of iodophors that varied considerably in their kill rates of bacteria and their duration of antibacterial activity. Iodophors tested in this study demonstrated a distinct superiority to noncomplexed iodine solutions (tincture and aqueous iodine solutions) as wound and skin cleansers.

Gram Stain

The purpose of this study was to identify pitfalls in the Gram staining technique that limit its diagnostic value. In our clinical experience, gram-positive organisms were often decolorized too easily. Factors have been identified that alter the susceptibility of gram-positive organisms to decolorization in the Gram staining technique. The age of the bacterial culture, the preparation of the smear, the fixation technique, and the mordant have an important influence on the ease with which gram-positive organisms are decolorized. On the basis of these studies, a more reliable and reporducible Gram staining technique has been developed for the diagnosis of surgical infections.

ADJUNCTIVE STUDIES

Skin Grafts

The purpose of this study was to identify deterimi-nants of split thickness skin graft infection. The bacterial count of the experimental wounds was proportional to the incidence of infection in split thickness skin grafts. When the wound was heavily contaminated with 10^7 organisms, infection developed under most grafts. Graft take frequently occurred in wounds subjected to a lower level of inoculum. The importance of bacterial counts as a determinant of potential skin graft infection was also suggested by a clinical study. We now routinely use quantitative bacterial counts to identify the granulating wounds that are ready for grafting.

The type of organism played no significant role in the development of infection.

The recipient site on which the graft was placed had an important bearing on infection of split thickness skin grafts. The incidence of skin graft infection was higher in fascial wounds than in dermal wounds contaminated with the same level of inoculum. Meshing of the split thickness skin graft offered no significant protection against infection. The infection rates of meshed and nonmeshed grafts did not differ significantly in experimental and clinical wounds.

Fingertip Amputation

Nonoperative management of fingertip pulp amputations has been employed in eighteen adults. After wound cleansing and debridement, the wound was covered by an occlusive dressing. Healing of the amputated fingertip occurred within four weeks. The healed fingertip had an excellent sensory perception, normal range of motion, and an acceptable cosmetic appearance. This satisfactory outcome was realized with less than ten days lost from work.

Cellulitis

Cellulitis of the scrotum and penis is caused, in the majority of instances, by a beta hemolytic streptococci without a discernible portal of entry. Clostridium, occasionally, will result in this disease as a manisfestation of a perirectal abscess. In either instance, fluid accumulates rapidly in the closed space between Colles' and

Buck's fascia, producing intense swelling of the scrotum. If this compartment is not immediately decompressed by linear incisions, devascularization of the scrotal and penile skin will often occur, resulting in gangrene. Immediate treatment of the bacterial infection with penicillin also is essential. If gangrene does develop, radical debridement of the necrotic tissue as well as a wide margin of adjacent inflamed skin must be undertaken. Continual monitoring of the microflora of the debrided wound is essential for the selection of the appropriate antibiotic against any secondary intruders. Coverage of the granulating wound is accomplished when the wound bacterial count is below 10^5 per gram of tissue.

Burn Management

A comprehensive picture of the burn wound microflora is now possible as a result of recent technologic advances. The microflora of the burn wound can be characterized with respect to its number, type, location, and antibiotic sensitivity. These parameters can be measured in sufficient time to influence the decision of the burn surgeon. The microflora of the surface of the burn wound and the burn wound itself are examined separately by different sampling techniques. For the surface microflora, the gauze capillary techniques employed, while incisional biopsies are used to monitor the organisms in the burn wound. After sampling, suspensions of the specimens are created which in turn are subjected to direct microscopic measurement,

quantitative culture procedures, and immediate antibiotic sensitivity testing. Histologic examination of the burn wound is performed concomitantly with this bacteriologic examination to determine the depth of bacterial invasion as well as to detect the presence of either mycotic or herpetic infections.

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